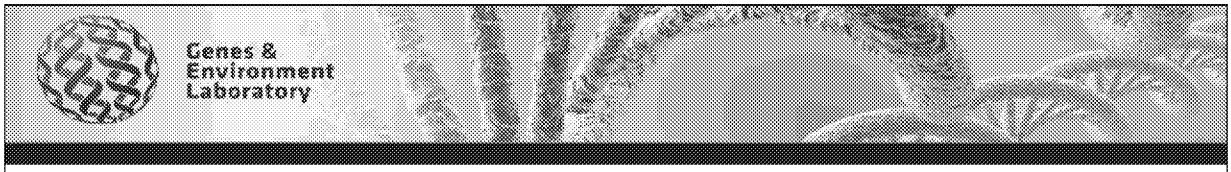

The key characteristics approach to evaluating mechanistic data in hazard identification and risk assessment

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UC BERKELEY
SUPERFUND
RESEARCH PROGRAM
SCIENCE FOR A SAFER WORLD

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Conflict of Interest Statement

- I am retained as a consultant and expert witness in U.S. litigation involving chemical and pharmaceutical exposures and various disease outcomes, including neuropathies and cancer, behalf of plaintiffs represented by Baron&Budd, Andrus-Wagstaff, the Metzger Law Group and the Locks Law Firm.

Conflict of Interest Statement, p.2

- I have no formal association with IARC, US EPA or CalEPA, but have an ongoing contract with OEHHA (Cal EPA) to further develop the key characteristics framework.
- The views expressed are solely my own.

KCs resulted from a large collaboration

- **IARC**: Kathryn Z. Guyton, Robert Baan and Kurt Straif
- **US EPA**: Catherine Gibbons, Jason Fritz, David DeMarini, Jane Caldwell, Robert Kavlock, Vincent Cogliano
- **NTP**: John Bucher **FDA**: Frederick Beland
- **Academia**: Ivan Rusyn, Paul F. Lambert, Stephen S. Hecht, Bernard W. Stewart, Weihsueh Chiu, Denis Corpet, Martin van den Berg, Matthew Ross, David Christiani
- **Consultant**: Christopher Portier
- **Acknowledgements:** Michele La Merrill for discussion and support from Research Translation Core of NIEHS SRP grant P42ES004705 and travel awards from IARC.

Summary of today's talk

- Scientific findings providing insights into cancer mechanisms play an increasingly important role in carcinogen hazard identification
- **The key characteristics of known human carcinogens provide the basis for a knowledge-based approach to evaluating mechanistic data rather than a hypothesis-based one like MOA/AOP**
- Shows carcinogens tend to act through multiple mechanisms in producing the hallmarks of human and animal tumors
- Recent IARC Monograph, EPA, CalEPA and NTP evaluations have illustrated the applicability of the KC approach
- May be compatible with HT assays, but need to develop new ones based on characteristics and hallmarks. Same for biomarkers.
- Key characteristics for other forms of toxicity are being developed

Integration of evidence to decide if a chemical is a human carcinogen?

- Human studies – epidemiology ⇓
- Animal studies – usually rodent bioassays – lifetime chronic ⇓ or shorter transgenic assays?
- In vitro studies ⇑ – e.g. Tox21/Toxcast
- Mechanistic data – Provides biological plausibility and increasing in importance

Who decides if a chemical is a carcinogen?

- International Agency for Research on Cancer (IARC –WHO) – Groups 1, 2A, 2B, 3, 4
- EPA – Groups A, B1, B2, C etc.
- NTP – Report on Carcinogens
- Cal Prop 65 – Often by adopting other authorities
- Others – FDA, EU, Japan etc.

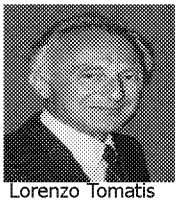
Definitions of the IARC Classifications

Classification	Definition
Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable as to its carcinogenicity to humans
Group 4	Probably not carcinogenic to humans

"The Encyclopaedia of Carcinogens"

Agents are recommended by international advisors based on:

- Evidence of human exposure
- Some evidence or suspicion of carcinogenicity



Lorenzo Tomatis
1929-2007

More than 980 agents have been evaluated

- 118 are ***carcinogenic to humans*** (Group 1)
- 79 are ***probably carcinogenic to humans*** (Group 2A)
- 290 are ***possibly carcinogenic to humans*** (Group 2B)
- 503 are ***not classifiable as to its carcinogenicity to humans*** (Group 3)
- 1 is classified as ***probably not carcinogenic to humans*** (Group 4)

National and international health agencies use the *Monographs*

- To identify carcinogens
- To prevent exposure to known or suspected carcinogens

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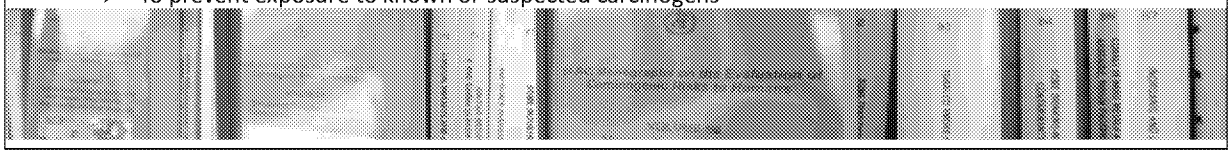
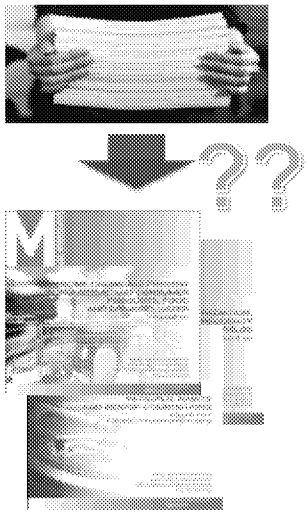
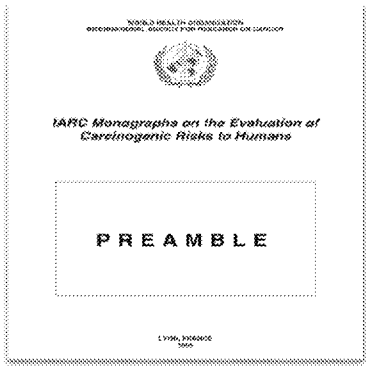
9



Soon after the IARC was founded 50 years ago, Lorenzo Tomatis had the great idea of creating a uniform classification system for carcinogens, based on objective criteria

How Are the IARC Monograph Evaluations Conducted?

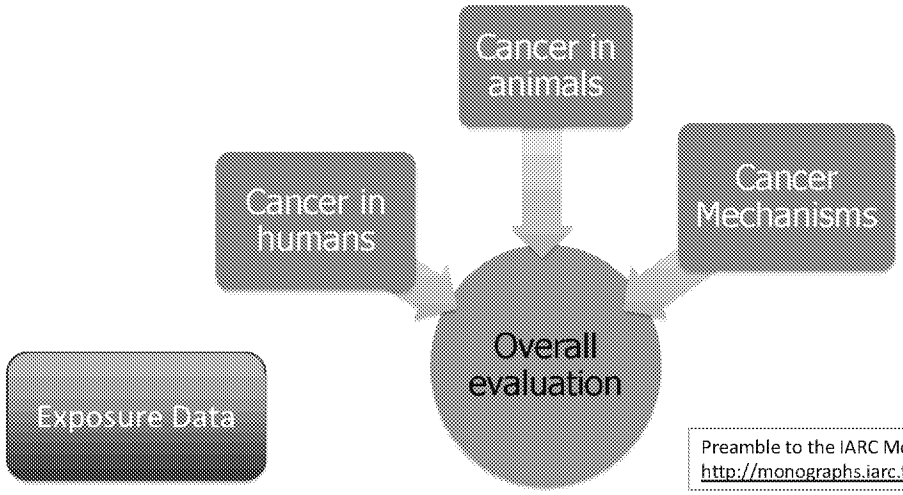




- Procedural guidelines for participant selection, conflict of interest, stakeholder involvement & meeting conduct
- Separate criteria for review of human, animal and mechanistic evidence
- Decision process for overall evaluations

Preamble to the IARC Monographs (2006):
http://monographs.iarc.fr/ENG/Preamble/index.php

What Evidence is Considered?



Preamble to the IARC Monographs (2006):
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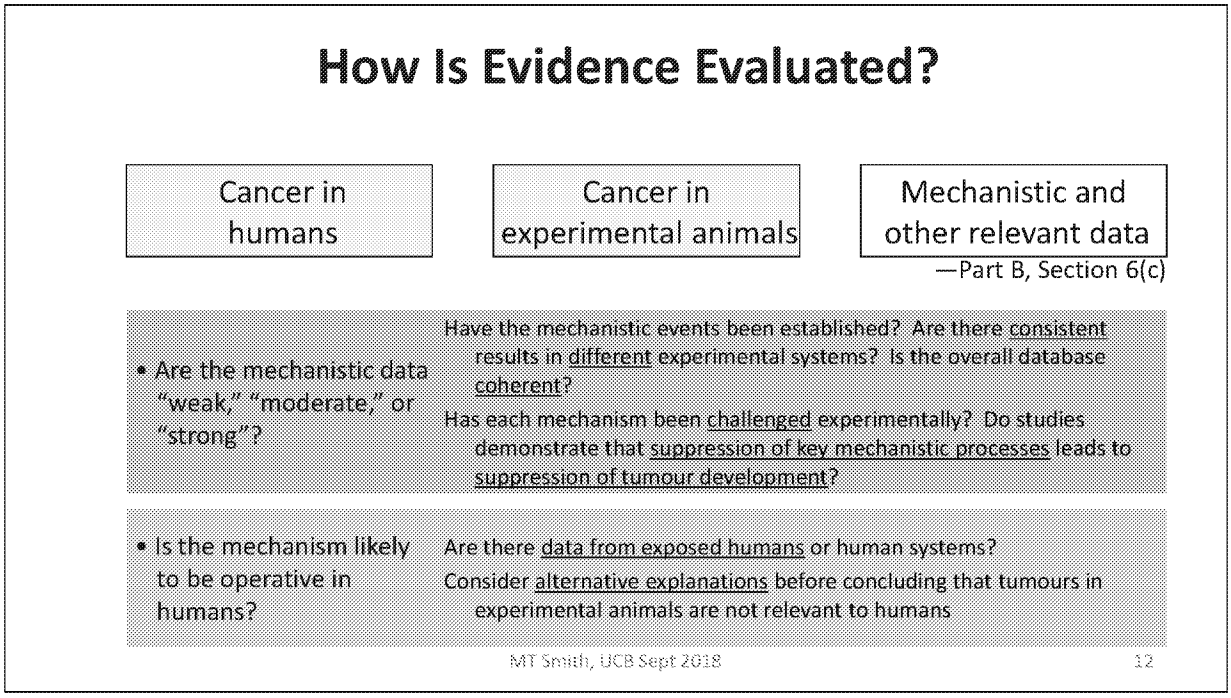
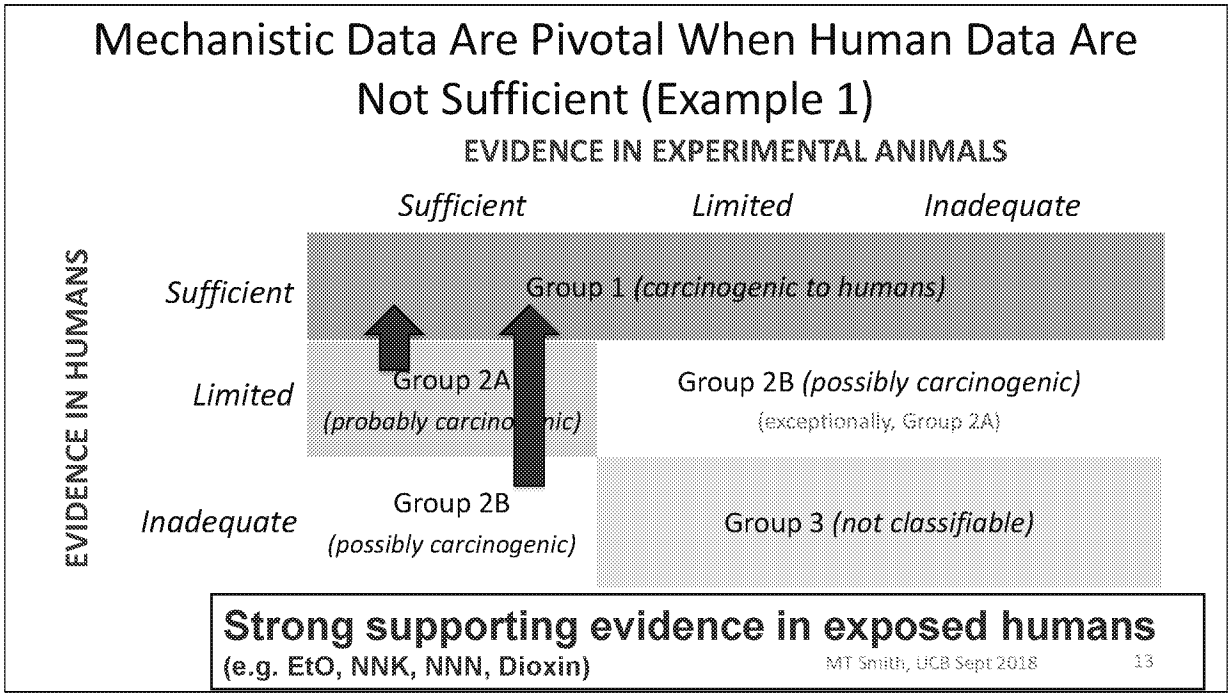


The categories for mechanistic data are strong, moderate or weak, reflecting the level of mechanistic support for a causal relationship. The evaluation of these data, as for studies of cancer in humans and animals, also involves ideas of consistency and coherence



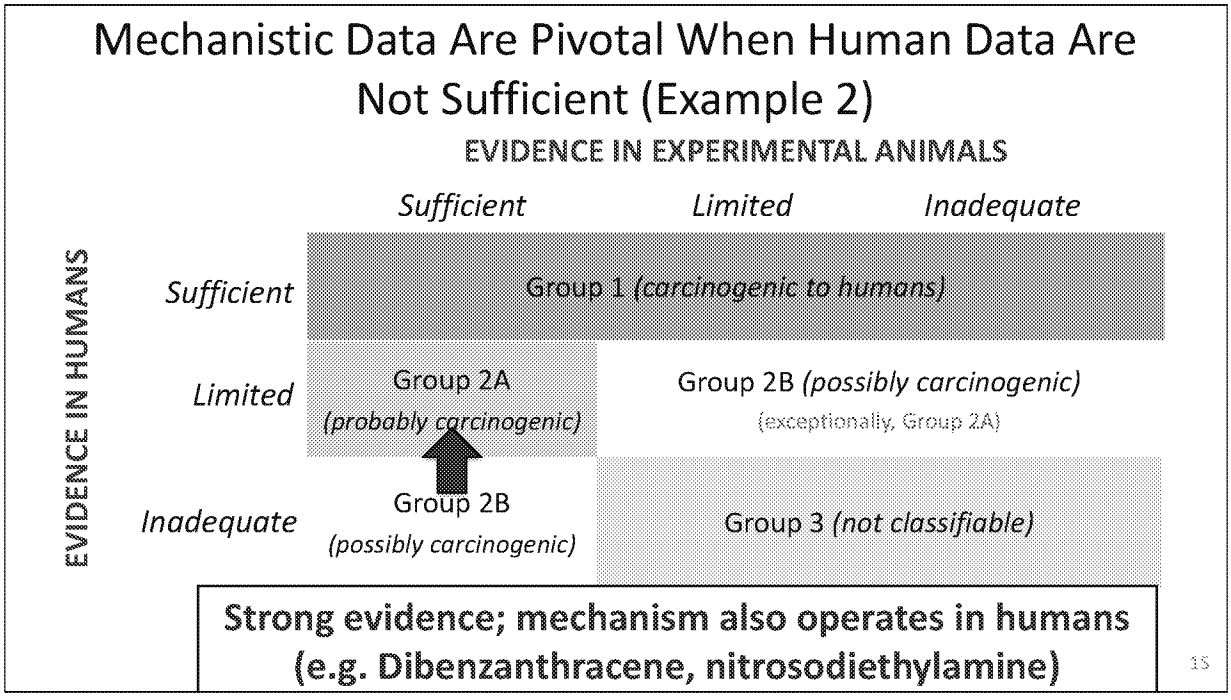
Mechanistic data are taken into account at the next stage: if the human data are less than sufficient, mechanistic evidence can modify the default evaluation based on human and animal data. The Preamble provides guidance for how this is done. For example, strong mechanistic evidence from studies of exposed humans can result in an upgrade to Group 1 from 2A or even 2B if there is sufficient evidence in animals.

IARC Group 1 Classifications Based on Different Mechanisms

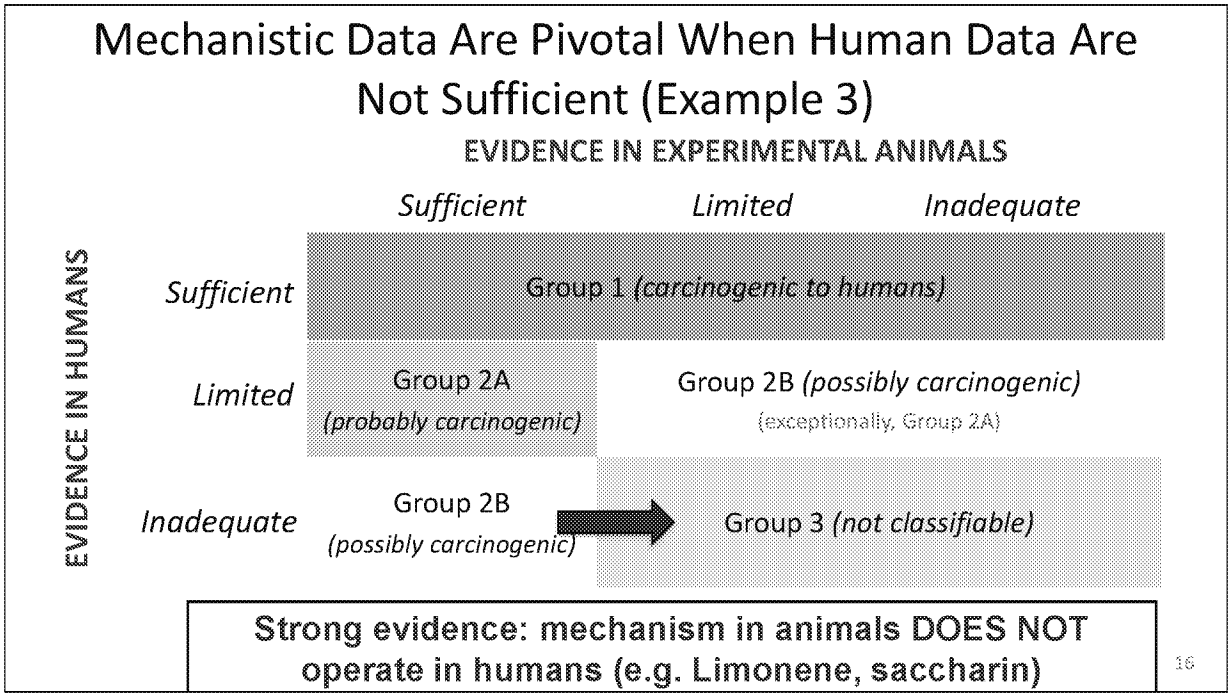
Agent	Mechanistic Rationale	Year (Vol)
Ethylene oxide	Genotoxic, cytogenetic effects in lymphocytes of workers	1994 (Vol 60)
NNN and NNK	Uptake, metabolism, DNA/haemoglobin adducts in smokeless tobacco users	2004 (Vol 89)

Agent	Mechanistic Rationale	Year
2,3,7,8-TCDD	Ah receptor binding, subsequent effects	1997 (Vol 69)

http://monographs.iarc.fr

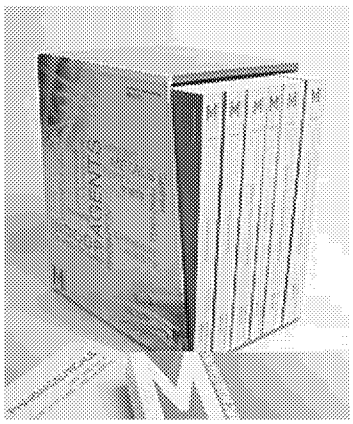


As another example, if there is sufficient evidence in animals and strong mechanistic evidence from experimental studies in animals or in vitro, but not in exposed humans, an upgrade from 2B to 2A is possible.



Finally, it's important to mention that a Group 2B agent classified only on the basis of sufficient animal data can be DOWNGRADED if there is strong evidence that the mechanism observed in animals doesn't operate in humans.

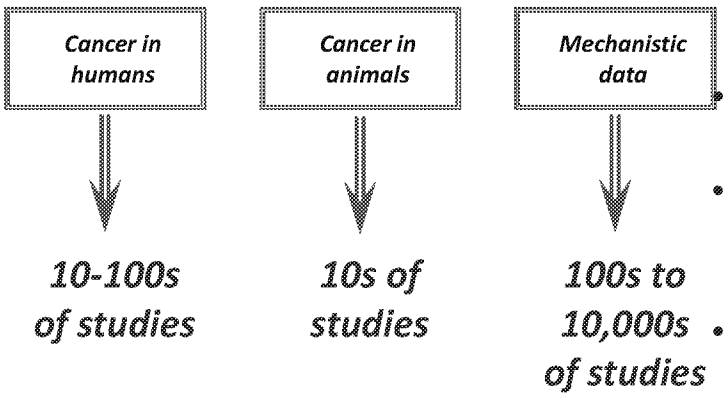
Mechanistic Data: *Challenges*



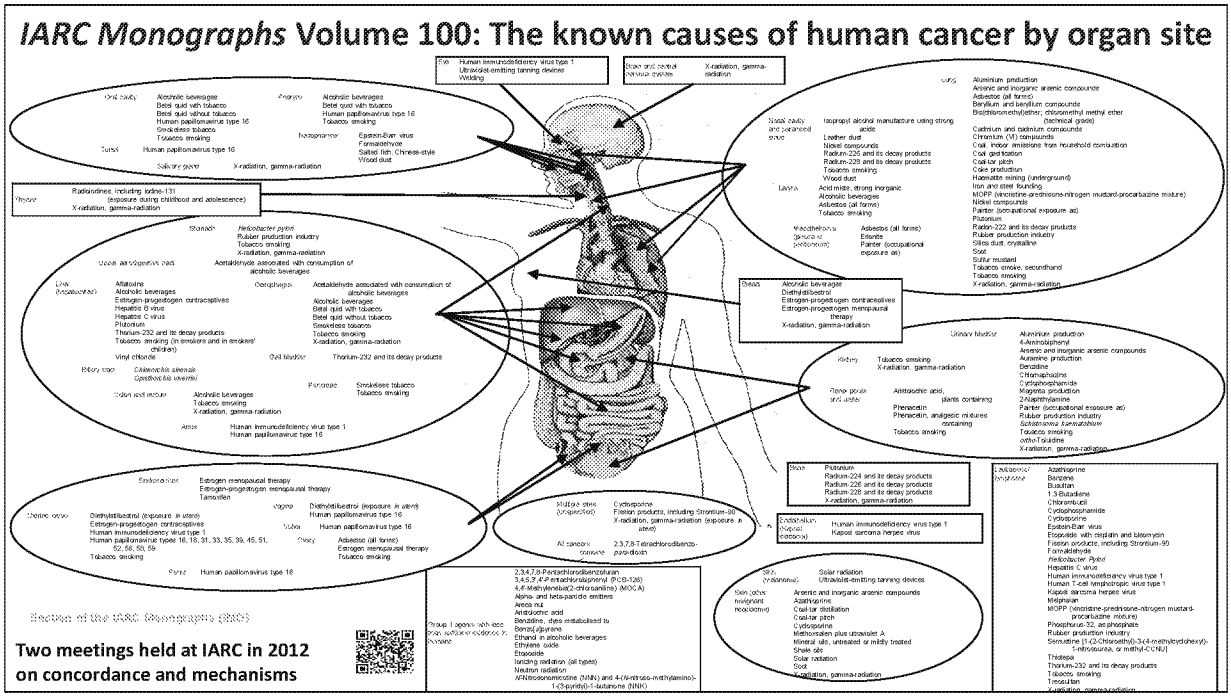
IARC Monographs
Volume 100

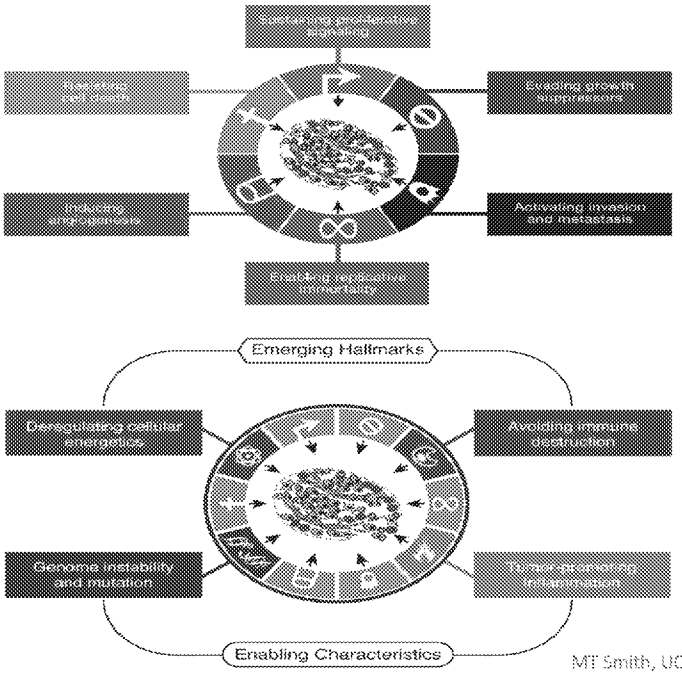
- Different human carcinogens may operate through distinct mechanisms
- Many human carcinogens act via multiple mechanisms
- There is no broadly accepted, systematic method for evaluating mechanistic data to support cancer hazard identification

So Many Studies, So Little Time…



- How to search systematically for relevant mechanisms?
- How to bring uniformity across assessments?
- How to analyze the voluminous mechanistic database efficiently?
- How to avoid bias towards favored mechanisms





HALLMARKS OF CANCER

1. Sustaining proliferative signaling
2. Evading growth suppressors
3. Resisting cell death
4. Enabling replicative immortality
5. Inducing aberrant angiogenesis
6. Activating invasion & metastasis

Emerging Hallmarks

- Reprogramming energy metabolism
- Evading immune destruction

Enabling Characteristics

- Genomic instability and mutation
- Inflammation

Hanahan and Weinberg 2011

Chemicals disrupt multiple hallmarks

Kleinstreuer N.C. et al. In vitro perturbations of targets in cancer hallmark processes predict rodent chemical carcinogenesis. Toxicol. Sci., (2013) 131, 40–55.

Chemical	HM1	HM2	HM3	HM4	HM5	HM6	HM7	HM8	HM9	HM 10	**TOTAL**
Chemical 1	X	X			X			X	X	X	**7**
Chemical 2			X	X			X				**3**
Chemical 3					X			X			**2**
Chemical 4	X	X		X			X	X	X		**6**

Tested 292 chemicals in 672 assays and successfully correlated the most disruptive chemicals (i.e. those that were most active across the various hallmarks) with known levels of carcinogenicity.

MT Smith, UCB Sept 2018 21

EPA tested a proposal for characterizing the carcinogenic potential of chemicals in humans, using in-vitro high-throughput screening (HTS) assays.
The selected HTS assays specifically matched key targets and pathways within the Hallmarks of Cancer framework.
The authors tested 292 chemicals in 672 assays and were successfully able to correlate the most disruptive chemicals (i.e. those that were most active across the various hallmarks) with known levels of carcinogenicity.

Chemicals were classified as 'possible'/'probable'/'likely' carcinogens or designated as 'not likely' or with 'evidence of non-carcinogenicity' and then compared with in-vivo rodent carcinogenicity data in the Toxicity Reference Database to evaluate their predictions. The model proved to be a good predictive tool, but it was developed only as a means to help the EPA prioritize many untested individual chemicals for their carcinogenic potential (i.e. in order to establish priorities for individual chemical testing (29)).

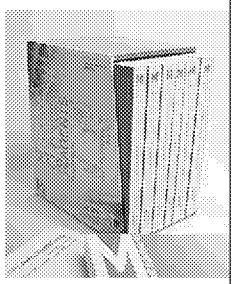
Multiple Mechanisms of Group 1 Carcinogens

[KZ Guyton….MT Smith, Mut Res 681; 230, 2009]

	Carcinogen			
Mechanisms	**Aflatoxin B1**	**Arsenic**	**Asbestos**	**Benzene**
DNA damage	+	+	-	+
Gene mutation	+	-	+	-
Chrom mutation	+	+	+	+
Aneuploidy	-	+	+	+
Epigenetic	+	+		+
Receptor signaling	-	+	+	
Other signaling	-	+		+
Immune effects	+	+	+	+
Inflammation	+	+	+	+
Cytotoxicity	+	+	+	+
Mitogenic	-	+		-
Gap junction	+	+		+

Dilemma: Cancer or Carcinogens

- Hallmarks are the biological characteristics of cancer cells and tumors in general, NOT the characteristic properties of human carcinogens
- Need to identify the key characteristics of human carcinogens
- IARC Working Group did this in 2012 and subsequently scientists at EPA, IARC and elsewhere determined how these characteristics could be searched for systematically



10 Key Characteristics of Human Carcinogens

Key characteristic:
1. Is electrophilic or can be metabolically activated
2. Is genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

- **Established human carcinogens** commonly exhibit one or more characteristics
- Data on these characteristics can **provide evidence of carcinogenicity**
- They can also **help in interpreting** the relevance and importance of findings of cancer in animals and in humans.

Smith MT, Guyton KZ, Gibbons CF, Fritz JM et al.. *Env Health Persp., 124(6):713-21*

Characteristic	Examples of relevant evidence
1. Is Electrophilic or Can Be Metabolically Activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.
2. Is Genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces Epigenetic Alterations	DNA methylation, histone modification, microRNA expression
5. Induces Oxidative Stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)

Characteristic	Examples of relevant evidence
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation, altered telomeres
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

A Hallmark *versus* a Key Characteristic

- A Hallmark describes what *IS*
- A Key Characteristic (KC) describes Something that makes "what is" happen

INTEGRATION OF THE KCs WITH HALLMARKS
Characteristics 1,2,4 and 8 can influence all Hallmarks

Key Characteristics

1. Is electrophilic or can be metabolically activated
2. Is genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

Hallmarks

1. Genetic Instability
2. Sustained Proliferative Signalling
3. Evasion of Anti-growth Signalling
4. Resistance to Cell Death
5. Replicative Immortality
6. Dysregulated Metabolism
7. Immune System Evasion
8. Angiogenesis
9. Inflammation
10. Tissue Invasion and Metastasis

PLUS - Tumor Microenvironment

MT Smith, UCB Sept 2018

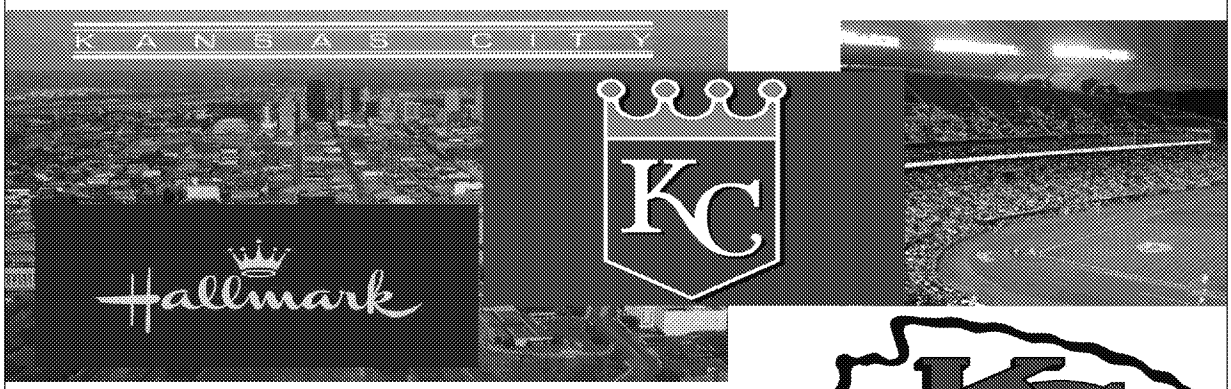
KCs act by disrupting Hallmark processes – Conclusion of Working Group convened in Berkeley, August 21-22, 2018

INTEGRATION OF THE KCs WITH HALLMARKS
Characteristics 3,5,6,7,9,10 influence specific Hallmarks

KC3: Alters DNA Repair or Causes Genomic Instability	(Hallmark) Genetic Instability
KC5: Induces Oxidative Stress	(Hallmark) Dysregulated Metabolism
KC6: Induces Chronic Inflammation	(Hallmark) Inflammation
KC7: Is Immunosuppressive	(Hallmark) Immune System Evasion
KC9: Causes Immortalization	(Hallmark) Replicative Immortality
KC10: Alters Cell Proliferation, Cell Death, or Nutrient Supply	(Hallmark) Sustained Proliferative Signalling (Hallmark) Evasion of Anti-growth Signalling (Hallmark) Resistance to Cell Death (Hallmark) Angiogenesis
NO KCs	(Hallmark) Tissue Invasion and Metastasis (Hallmark) Tumor Microenvironment

Several KCs act by disrupting specific Hallmark processes – From Leroy Lowe's presentation to Working Group convened in Berkeley, August 21-22, 2018

According to Bill Goodson from Kansas City the KCs were bound to integrate with the Hallmarks



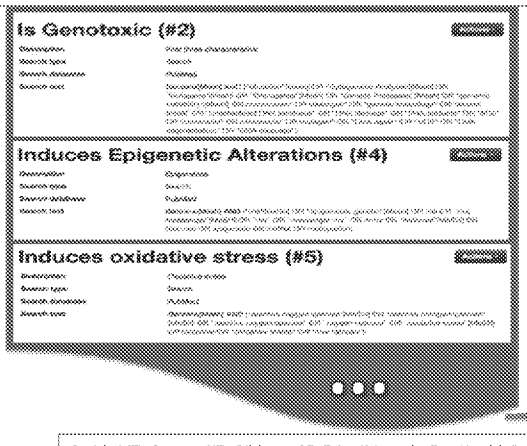
Exception: KC and the Sunshine Band are from Florida

Applications of the KCs

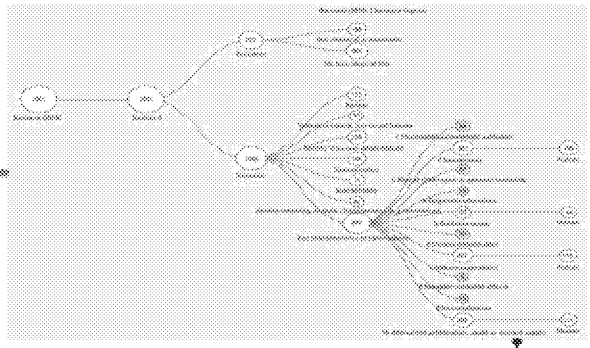
- Searching the literature – Set of MeSH terms developed – Facilitate systematic review
- Identify data gaps
- Development of MOA/AOP or networks
- Improve predictive toxicology
- Better understanding of cumulative risk

Systematic Approach
Using Key Characteristics of Carcinogens

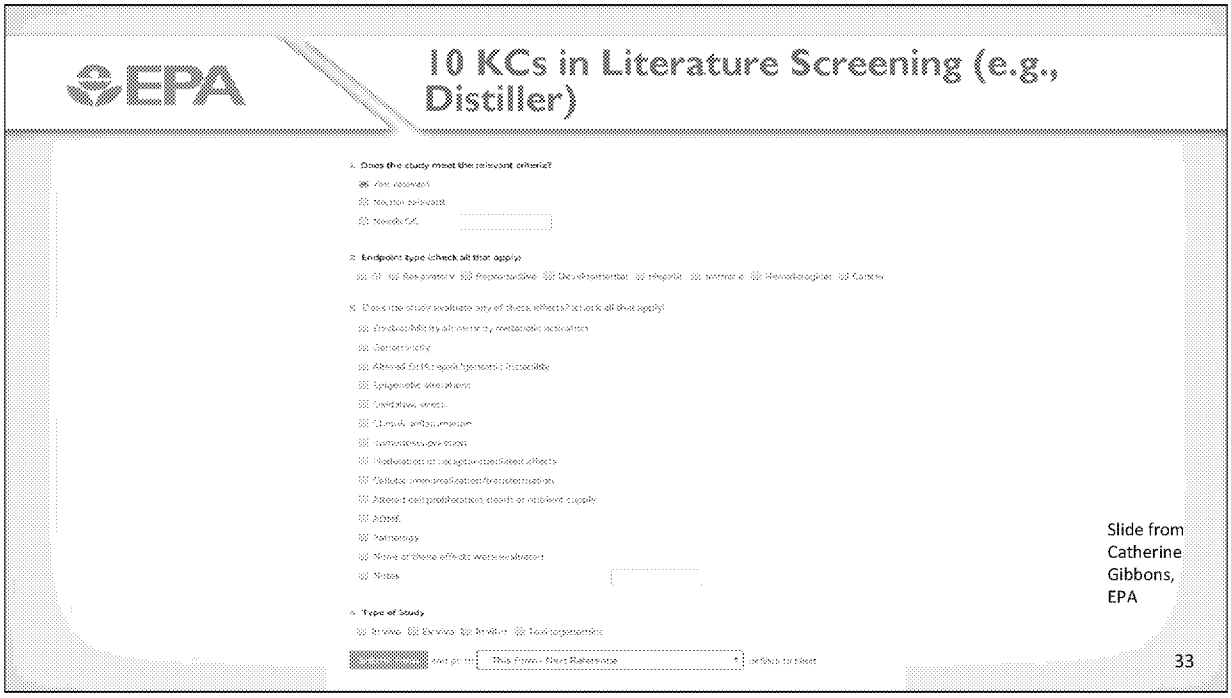
Targeted searches for each key characteristic



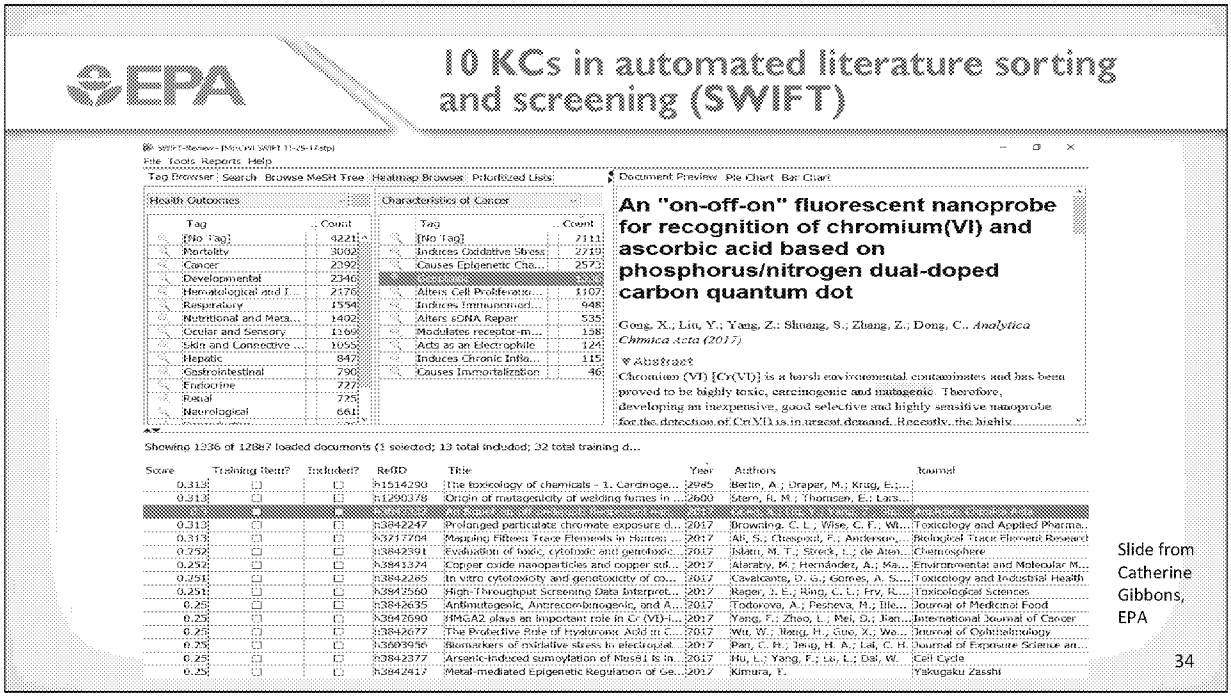
Organize results by key characteristics, species, etc



Smith MT, Guyton KZ, Gibbons CF, Fritz JM et al.. *Env Health Persp.*, *124(6):713-21*



10 KCs provide tags for preliminary screening; we are working on a list of standardized, customizable tags based on commonly associated endpoints and assays for each characteristic (similar to IARC's list) to provide another level of screening. This is a screenshot from Distiller, but HAWC is also effective for screening, though Distiller records selections from multiple reviewers and identifies conflicts. We primarily use HAWC to record study evaluation decisions, to extract data from human and animal studies, and to create tables and visualizations; right now, we don't have immediate plans to extend this to mechanistic/in vitro studies.



Slide from Catherine Gibbons, EPA

34

SWIFT uses machine learning approaches that will sort studies by key characteristic, based on looking at existing searches used by IARC, RoC, and working with an NIEHS information scientist, but these are a few years old and need to be updated and optimized, which we will work with them on. Right now it is most useful for getting a general idea of what a database looks like, or to identify and prioritize specific studies of a given type in a database, allowing the user to "teach" the program what studies are most relevant while screening. SWIFT does offer a lot of flexibility, the pre-set searches in SWIFT Review can be adjusted by the user.

Application of the KCs at IARC

Use the KCs to:

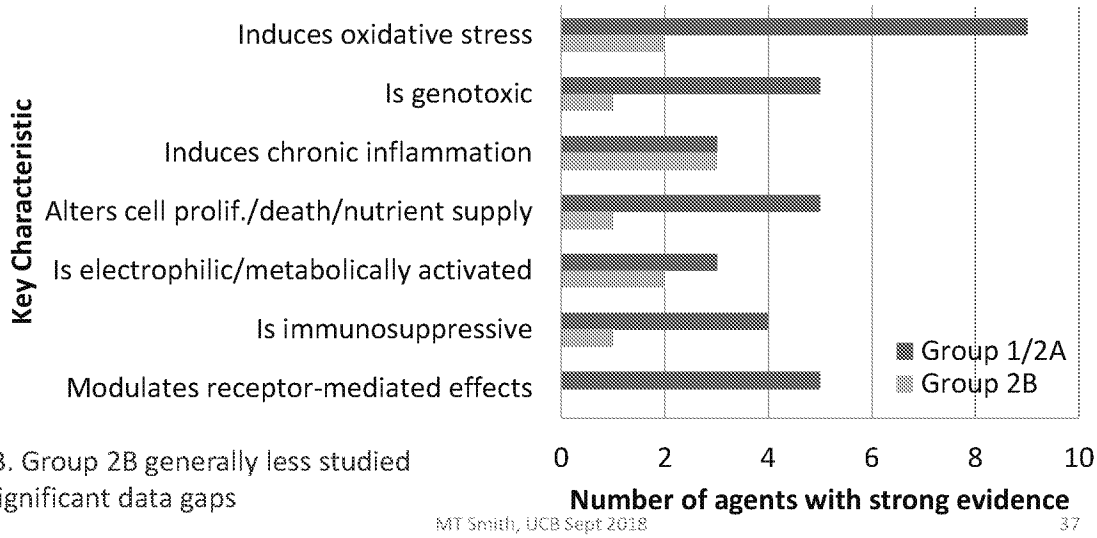
- Identify the relevant mechanistic information
- Screen and organize the search results
- Evaluate quality of the identified studies
- Summarize the evidence for each KC as strong, moderate or weak and determine if it operates in humans or human in vitro systems

Use of KCs in Recent IARC Monographs Evaluations

Agent	Group	Cancer in humans	Cancer in animals	Strong mechanistic evidence (key characteristic)
Penta-chlorophenol	1	**Sufficient**	Sufficient	Is metabolically activated, is genotoxic, induces oxidative stress, modulates receptor-mediate effects, alters cell proliferation or death (1, 2, 5, 6, 8, 10)
Welding fumes	1	**Sufficient**	Sufficient	Are immunosuppressive, induce chronic inflammation (6, 7)
DDT	2A	**Limited**	**Sufficient**	Modulates receptor-mediated effects, is immunosuppressive, induces oxidative stress (5,7,8)
Dimethyl-formamide	2A	**Limited**	**Sufficient**	Is metabolically activated, induces oxidative stress, alters cell proliferation (1, 5, 10)
Tetrabromo-bisphenol A	2A*	Inadequate	**Sufficient**	Modulates receptor-mediated effects, is immunosuppressive, induces oxidative stress (5, 7, 8)
Tetrachloro-azobenzene	2A*	Inadequate	**Sufficient**	Induces oxidative stress, is immunosuppressive, modulates receptor-mediated effects (6, 8, 10)
ITO, melamine	2B	Inadequate	**Sufficient**	Induces chronic inflammation (8)
Parathion, TCP	2B	Inadequate	**Sufficient**	

*Overall evaluation upgraded to Group 2A with supporting evidence from other relevant data

Key Characteristics with Strong Evidence across Multiple Evaluations (IARC Monographs Vol. 112-119)



N.B. Group 2B generally less studied – significant data gaps

Applications of the KCs

- Searching the literature – Set of MeSH terms developed – Facilitate systematic review
- Identify data gaps
- Development of MOA/AOP or networks
- Improve predictive toxicology
- Better understanding of cumulative risk

Use of the KCs by the NTP Report on Carcinogens

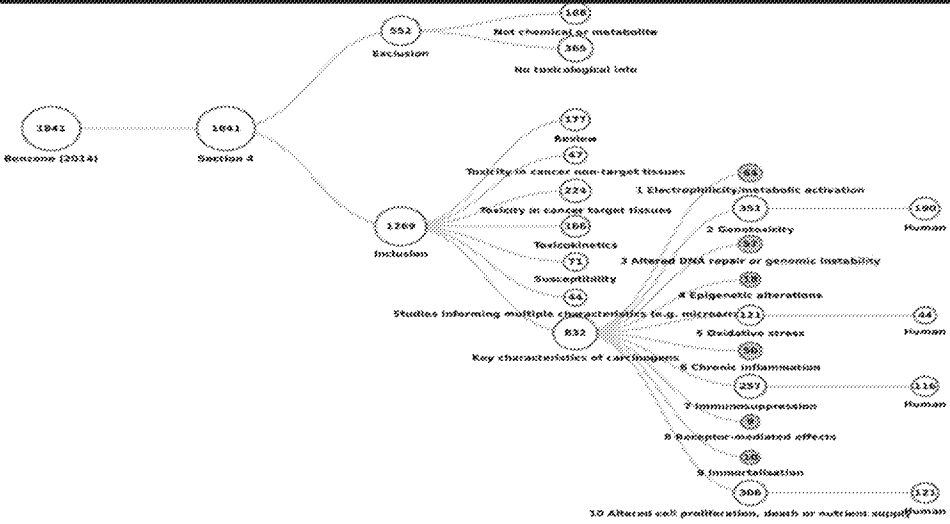
Table 6-4. Possible modes of carcinogenic action for haloacetic acids and the 10 characteristics of carcinogens

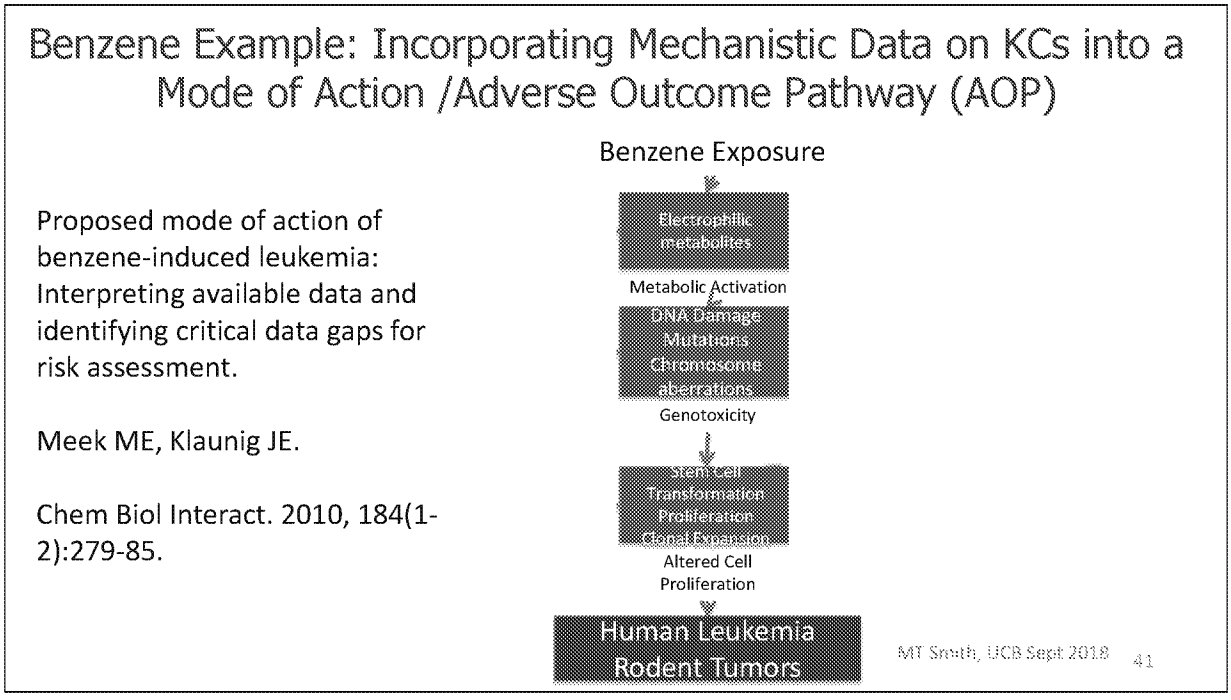
Characteristic(s) of carcinogens	Mode of action	Key events
Electrophilicity	Irreversible binding to macromolecules	1. Haloacetic acids have an electrophilic structure that can react with peptides, proteins, or DNA to form adducts. 2. Protein or DNA adducts result in altered activity or DNA damage that advances acquisition of multiple critical traits contributing to carcinogenesis.
Altered nutrient supply, electrophilicity, induction of oxidative stress	Reprogramming cellular energy metabolism (inhibition of pyruvate dehydrogenase kinase (PDK)	1. Haloacetic acids inhibition of PDK increases pyruvate dehydrogenase complex activity and oxidative metabolism. 2. Increase in oxidative metabolism leads to an increase in reactive oxygen species (ROS) and oxidative stress. 3. Oxidative stress leads to acquisition of multiple, critical traits contributing to carcinogenesis.
Altered nutrient supply, electrophilicity, induction of oxidative stress	Inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1. Haloacetic acids inhibition of GAPDH leads to inhibition of glycolysis. 2. Inhibition of glycolysis leads to reduced ATP levels and repressed pyruvate generation. 3. Reduced pyruvate leads to mitochondrial stress, ROS generation, cytotoxicity, and DNA damage.
Induction of oxidative stress	Oxidative stress	1. Haloacetic acids induce oxidative stress through multiple pathways. 2. Oxidative stress can cause mutations and damage to proteins, lipids, and DNA. 3. Mutations and damage to macromolecules activate cell-signaling pathways, induce genomic instability, and cell transformation and lead to cancer.

https://ntp.niehs.nih.gov/ntp/about_ntp/monopeerrvw/2017/july/haafinalmonograph_508.pdf

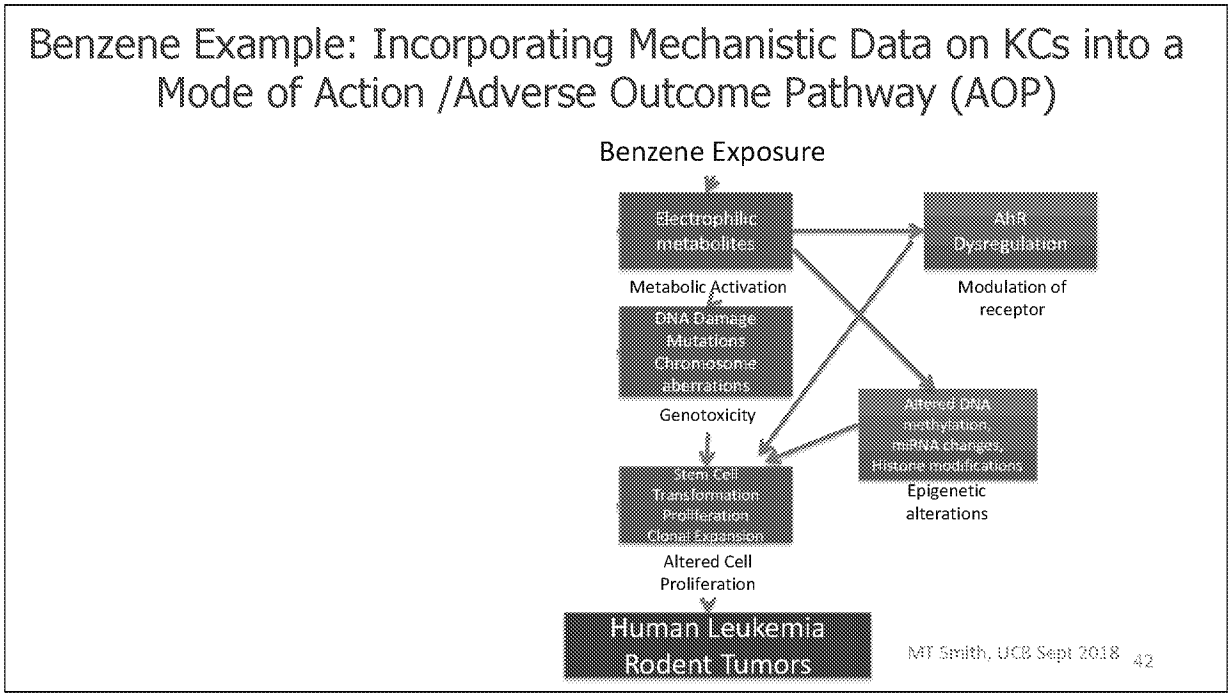
Benzene Mechanistic Data Search

conducted using the Health Assessment Workplace Collaborative (HAWC) Literature Search tool (https://hawcproject.org/)





An overview of how benzene induces 8 of the key characteristics in a probable mechanism of carcinogenicity. A full review of these mechanistic data is given in (McHale et al. 2012), from which this Figure was adapted



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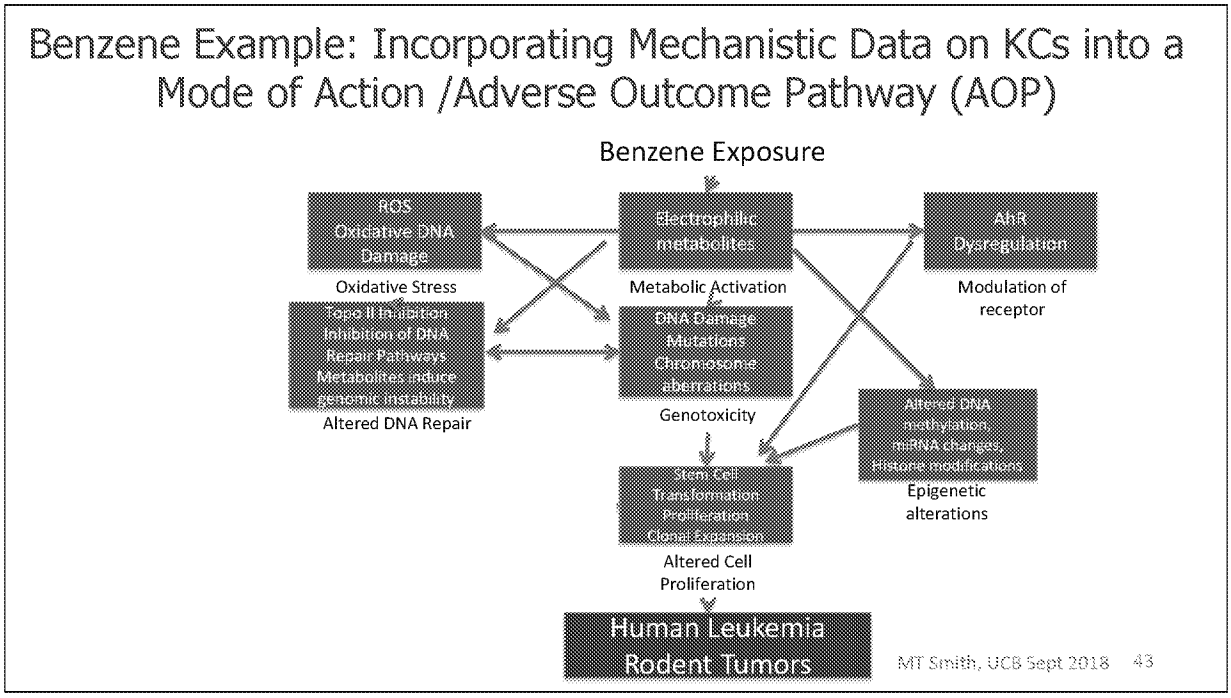

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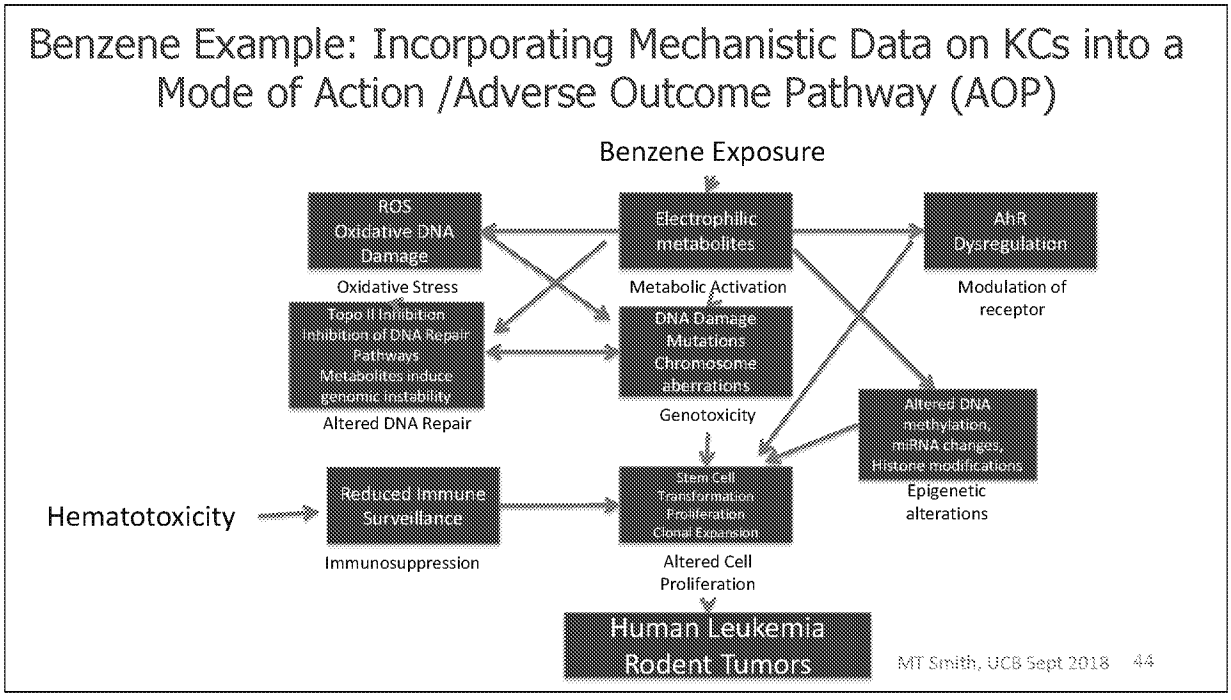

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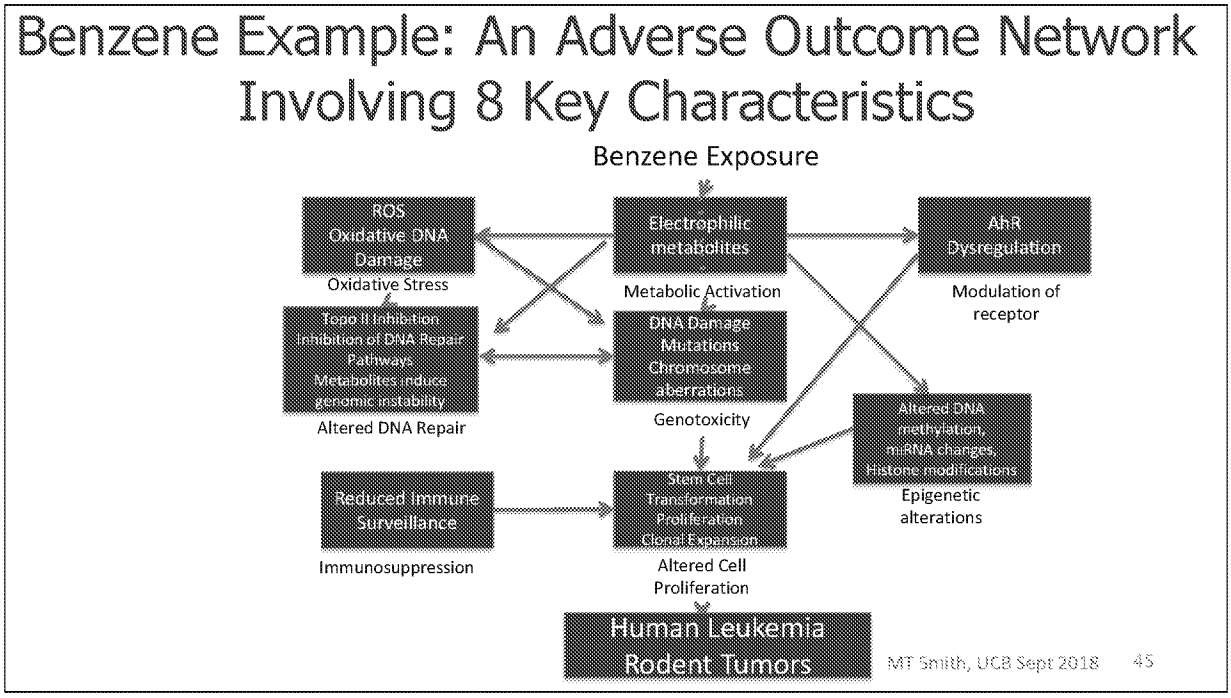

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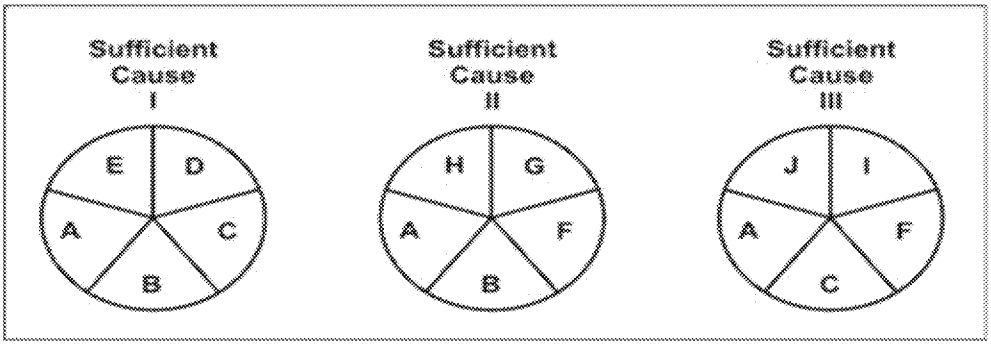
Limitations of MOA/AOP Approach

- Biology is not linear – influenced by feedback mechanisms, repair, background, susceptibilities…Network of systems
- Multiple ways to arrive at same conclusion – Does not fit with Causal Pie concept
- Limited by the current understanding of the disease process (recognized by Sir Bradford Hill, who noted that "what is biologically plausible depends upon the biological knowledge of the day")
- Key events are supposed to be quantifiable but in reality they may be impossible to measure

Rothman's Causal Pies

Three causal pies each with various components.



MOA/AOP approach does not fit with Rothman's causal pies concept which envisages multiple combinations of causes producing a disease

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Limitations of MOA/AOP Approach (continued)

- MOA/AOP may be incomplete or wrong [e.g. DEHP – Rusyn and Corton (2012)]
- Focus on 'favorite' mechanism may introduce bias, especially on committees and public databases
- How many 'validated' AOPs needed for 100K chemicals producing 100s of adverse outcomes in different ways?

Key characteristics don't require risk assessor to guess the mechanism

- Mechanistic hypotheses in science are beneficial because if you test it and are wrong then you modify the hypothesis and get closer to the truth

- Mechanistic hypotheses in risk assessment are problematic because if you are wrong you may have made a bad risk decision that cannot easily be changed and may have caused medical or economic harm

New National Academy of Sciences report released January 5, 2017

https://www.nap.edu/download/24635

Using 21st Century Science to Improve Risk-Related Evaluations

260 pages | 6 x 9 | PAPERBACK
ISBN 978-0-309-45348-6 | DOI: 10.17226/24635

AUTHORS

Committee on Incorporating 21st Century Science into Risk-Based Evaluations; Board on Environmental Studies and Toxicology; Division on Earth and Life Studies; National Academies of Sciences, Engineering, and Medicine

Using 21st Century Science to Improve Risk-Related Evaluations - Comments

- The KC "approach avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence." (P.144)
- "The committee notes that key characteristics for other hazards, such as cardiovascular and reproductive toxicity, could be developed as a guide for evaluating the relationship between perturbations observed in assays, their potential to pose a hazard, and their contribution to risk." (p.141)
- Through a project funded by OEHHA (Cal EPA), KCs for reproductive toxicants and endocrine disruptors have been developed

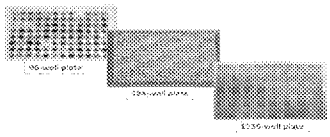
Working Group on KCs of Endocrine Disruptors and Reproductive Toxicants

Berkeley CA, March 7-8, 2018

Using 21st Century Science to Improve Risk-Related Evaluations - Recommendation

"The committee encourages the cataloging of pathways, components, and mechanisms that can be linked to particular hazard traits, similar to the IARC characteristics of carcinogens. This work should draw on existing knowledge and current research in the biomedical fields related to mechanisms of disease that are outside the traditional toxicant-focused literature that has been the basis of human health risk evaluations and of assessments and toxicology. The work should be accompanied by research efforts to describe the series of assays and responses that provide evidence on pathway activation and to establish a system for interpreting assay results for the purpose of inferring pathway activation from chemical exposure." (p.156)

ToxCast Assays (>800 endpoints)



Assay Provider
ACEA
Apredica
Attagene
BioReliance
BioSeek
CeeTox
CellzDirect
Tox21/NCATS
NHEERL MESC
NHEERL Zebrafish
NovaScreen (Perkin Elmer)
Odyssey Thera
Vala Sciences

Biological Response
cell proliferation and death
cell differentiation
Enzymatic activity
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor binding
receptor activity
steroidogenesis

Target Family
TF response element
transporter
cytokines
kinases
nuclear receptor
CYP450 / ADME
cholinesterase
phosphatases
proteases
XME metabolism
GPCRs
ion channels

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type
single
multiplexed
multiparametric

Cell Format
cell free
cell lines
primary cells
complex cultures
free embryos

Species
human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source

Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
ELISA

List of assays, data, and related information at: **http://www.epa.gov/ncct/**

High-Throughput Screening Data

ToxCast iCSS dashboard
(http://actor.epa.gov/dashboard/)

- 821 assays
- 1860 chemicals

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10 Key Characteristics of Human Carcinogens
1. Is electrophilic or can be metabolically activated
2. Is genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

= ??

At most, 274 ToxCast/Tox21 assays could be mapped to a key characteristic:

Key Characteristic	1. Is electrophilic or can be metabolically activated	4. Induces epigenetic alterations	5. Induces oxidative stress	6. Induces chronic inflammation	8. Modulates receptor-mediated effects	10. Alters cell proliferation, cell death and nutrient supply
Assay Endpoints	31 assays: • CYP inhibition (29) • Aromatase inhib. (2)	11 assays: • DNA binding (4) • Transformation (7)	18 assays: • Metalloproteinase (5) • Oxidative stress (7) • Oxidative stress marker (6)	45 assays: • Cell adhesion (14) • Cytokines (29) • NFkB (2)	81 assays: • AhR (2) • AR (11) • ER (18) • FXR (7) • Others (18) • PPAR (12) • PXR_VDR (7) • RAR (6)	68 assays: • Cell cycle (16) • Cytotoxicity (41) • Mitochondrial toxicity (7) • Proliferation (4)

No assay coverage for 4 key characteristics

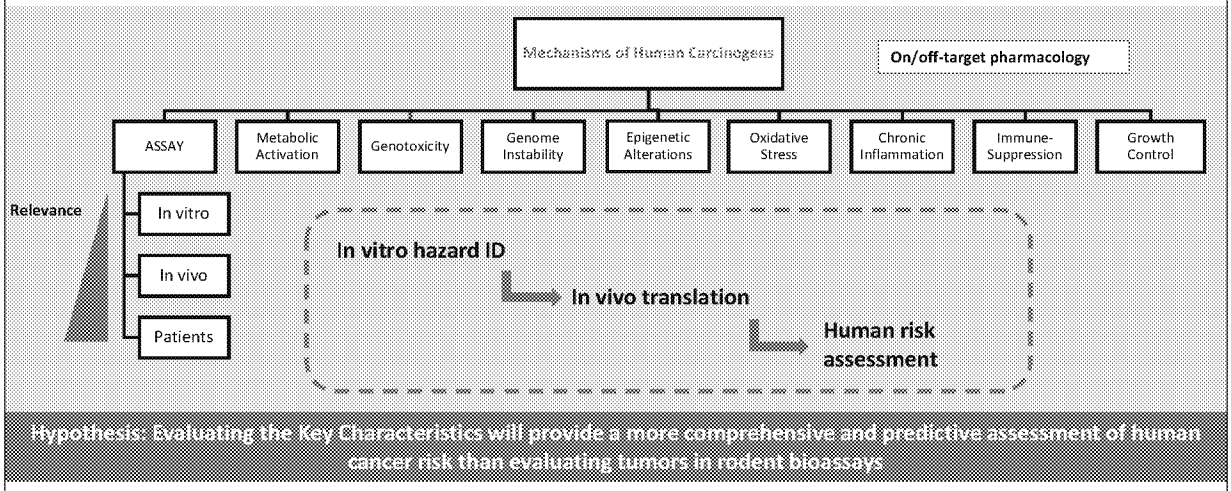
2. Is Genotoxic	3. Alters DNA repair or causes genomic instability	7. Is immunosuppressive	9. Causes immortalization

Chiu WA, Guyton KZ, Martin MT, Reif DM, Rusyn I. *ALTEX.*, PMID: 28738424.

What Next for the Key Characteristics?

- Refinement of definitions and listing of all assays for each characteristic
- Development of HT assays specific for each characteristic – A CarciCAST – Testing of new drugs and chemicals (see Fielden et al. 2017)
- Key characteristics of other endpoints – cardiovascular toxicity; developmental toxicity etc.

Use of KC's for assessment of therapeutics



Hypothesis: Evaluating the Key Characteristics will provide a more comprehensive and predictive assessment of human cancer risk than evaluating tumors in rodent bioassays

Growth control: proliferation, apoptosis, immortalization, metabolism

Adapted from Fielden et al Trends Pharmacol Sci. 2018

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Question for the Future

If a chemical possesses multiple key characteristics can we classify it as a possible/probable human carcinogen without any animal bioassay or epidemiological data?

Summary

- Scientific findings providing insights into cancer mechanisms play an increasingly important role in carcinogen hazard identification
- **The key characteristics of known human carcinogens provide the basis for a knowledge-based approach to evaluating mechanistic data rather than a hypothesis-based one like MOA/AOP**
- Shows carcinogens tend to act through multiple mechanisms in producing the hallmarks of human and animal tumors
- Recent IARC Monograph, EPA, CalEPA and NTP evaluations have illustrated the applicability of the KC approach
- May be compatible with HT assays, but need to develop new ones based on characteristics and hallmarks. Same for biomarkers.
- Key characteristics for other forms of toxicity are being developed


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